

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
27 September 2001 (27.09.2001)

PCT

(10) International Publication Number
WO 01/071036 A3

- (51) International Patent Classification⁷: **C12Q 1/68**, **C12N 15/10**
- (21) International Application Number: **PCT/US01/08501**
- (22) International Filing Date: **16 March 2001 (16.03.2001)**
- (25) Filing Language: **English**
- (26) Publication Language: **English**
- (30) Priority Data:
60/190,056 17 March 2000 (17.03.2000) US
09/669,739 26 September 2000 (26.09.2000) US
- (71) Applicant (for all designated States except US): **GENE LOGIC, INC.** [US/US]; 708 Quince Orchard Road, Gaithersburg, MD 20878 (US).
- (72) Inventors; and
- (75) Inventors/Applicants (for US only): **EASTMAN, Eric** [US/US]; c/o Gene Logic, Inc., 708 Quince Orchard Road, Gaithersburg, MD 20878 (US). **HARTWELL, John** [US/US]; c/o Gene Logic, Inc., 708 Quince Orchard Road, Gaithersburg, MD 20878 (US). **MILLSTEIN, Larry** [US/US]; c/o Gene Logic, Inc., 708 Quince Orchard Road, Gaithersburg, MD 20878 (US). **KUZIORA, Michael** [US/US]; c/o Gene Logic, Inc., 708 Quince Orchard Road, Gaithersburg, MD 20878 (US). **GUILFOYLE, Richard** [US/US]; c/o Gene Logic, Inc., 708 Quince Orchard Road, Gaithersburg, MD 20878 (US). **HOKE, Glenn** [US/US]; c/o Gene Logic, Inc., 708 Quince Orchard Road, Gaithersburg, MD 20878 (US).
- (74) Agents: **TUSCAN, Michael, S. et al.**; Morgan, Lewis & Bockius LLP, 1111 Pennsylvania Avenue, NW, Washington, DC 20004 (US).
- (81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.
- (84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).
- Published:**
— with international search report
— before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments
- (88) Date of publication of the international search report:
24 October 2002
- For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

(54) Title: METHODS OF PREPARING AMPLIFIED NUCLEIC ACID MOLECULES

(57) Abstract: New and improved methods are provided for generating amplified nucleic acid molecules from cellular mRNA. The methods are robust and reliable, and can be used to provide gene fragments for use in methods of analyzing gene expression patterns. The methods comprise: (a) preparing double-stranded cDNA by: (i) hybridizing at least one primer comprising an RNA polymerase promoter to said population of RNA molecules and extending said primer by reverse transcription to generate single-stranded cDNA, and (ii) synthesizing double-stranded cDNA from said single-stranded cDNA by priming with an oligonucleotide mixture having a random sequence selected from the group consisting of a tetramer oligonucleotide mixture, a pentamer oligonucleotide mixture, a hexamer oligonucleotide mixture, a heptamer oligonucleotide mixture, an octamer oligonucleotide mixture, a nonamer oligonucleotide mixture, a decamer oligonucleotide mixture and mixtures thereof; and (b) transcribing amplified copies of anti-sense RNA from said double-stranded cDNA.

WO 01/071036 A3

PCT/US 01/0

IPC 7 C1201/68 C12N15/10

B. FIELDS SEARCHED

IPC 7 C120 C12N

EPO-Internal, BIOSIS, PAJ, MEDLINE, SEQUENCE SEARCH

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
------------	--	-----------------------

Y	US 5 514 545 A (EBERWINE JAMES) 7 May 1996 (1996-05-07) the whole document ---	1-20
Y	US 5 891 636 A (BARCHAS JACK D ET AL) 6 April 1999 (1999-04-06) the whole document ---	1-20
Y	US 5 851 805 A (MCCORMICK JOSEPH JUSTIN ET AL) 22 December 1998 (1998-12-22) column 10 ---	1-20
A	US 5 665 547 A (LIANG PENG ET AL) 9 September 1997 (1997-09-09) the whole document ---	1-20
-/--		

Y Patent family members are listed in annex.

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "Z" document member of the same patent family

Date of mailing of the international search report

26/08/2002

Reuter, U

INTERNATIONAL SEARCH REPORT

International Application No.

PCT/US 01/00001

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 97 27317 A (CHEE MARK ;LAI CHAOQIANG (US); LEE DANNY (US); AFFYMETRIX INC (US)) 31 July 1997 (1997-07-31) example 14 -----	1-20
A	DATABASE BIOSIS 'Online! BIOSCIENCES INFORMATION SERVICE, PHILADELPHIA, PA, US; 1989 FRIEMERT C ET AL: "PREPARATION OF RADIOLABELED COMPLEMENTARY DNA PROBES WITH HIGH SPECIFIC ACTIVITY FOR RAPID SCREENING OF GENE EXPRESSION" Database accession no. PREV199192135875 XP002209828 abstract & METHODS IN MOLECULAR AND CELLULAR BIOLOGY, vol. 1, no. 4, 1989, pages 143-154, ISSN: 0898-7750 -----	1-20

INTERNATIONAL SEARCH REPORT

International Application
PCT/US 01/08301

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
US 5514545	A	07-05-1996	NONE	
US 5891636	A	06-04-1999	US 5716785 A US 5545522 A US 6291170 B1	10-02-1998 13-08-1996 18-09-2001
US 5851805	A	22-12-1998	NONE	
US 5665547	A	09-09-1997	US 5599672 A US 5262311 A US 5965409 A CA 2102784 A1 EP 0592626 A1 JP 2843675 B2 JP 7500735 T WO 9318176 A1	04-02-1997 16-11-1993 12-10-1999 12-09-1993 20-04-1994 06-01-1999 26-01-1995 16-09-1993
WO 9727317	A	31-07-1997	AU 2253397 A EP 0880598 A1 JP 2002515738 T WO 9727317 A1 US 6344316 B1	20-08-1997 02-12-1998 28-05-2002 31-07-1997 05-02-2002